Isolation and Characterization of Novel 2-Hydroxy Fatty Acids from the Phospholipids of the Sponge *Smenospongia aurea*

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The Caribbean sponge *Smenospongia aurea* revealed the presence of six novel branched α-hydroxy fatty acids: 2-hydroxy-17-methyloctadecanoic acid, 2-hydroxy-21-methyldocosanoic acid, 2-hydroxy-22-methyltricosanoic acid, 2-hydroxy-22-methyltetracosanoic acid, 2-hydroxy-24-methylpentacosanoic acid, and 2-hydroxy-23-methylpentacosanoic acid. These novel α-hydroxy fatty acids were associated with phosphatidylethanolamine. The sponges *Aplysina lacunosa* and *Aplysina fistularis* also contained considerable amounts of α-hydroxy fatty acids, the very long-chain 5,9,23-tricontatrienoic acid (30:3), and phytanic acid. The sterol composition of the three sponges was also studied. It indicated that *A. lacunosa* and *A. fistularis* contained large amounts of aplysterol and verongulasterol, while *S. aurea* did not show any of these sterols. The results are discussed in terms of the taxonomy of the species.
***Lipids* 27, 681–685 (1992).**

Sponges belonging to the genus *Smenospongia* and *Aplysina* have shown interesting morphological similarities that warrant a closer scrutiny of their secondary metabolites (1). The Caribbean sponge *Smenospongia aurea* (Hyatt, 1875) contains the 5-bromo- and 5,6-dibromo-*N,N*-dimethyltryptamines, aureol, and 6-bromoaplysinopsin as well as 6-bromo-4′-*N*-demethylaplysinopsin (1). The *Aplysina* species, identified as *Aplysina lacunosa* (Pallas, 1766) and *Aplysina archeri* (Higgin, 1875), were also shown to contain mixtures of these metabolites and/or bromotyrosine derived compounds. An earlier report indicated similar results for *S. aurea* and *Smenospongia (= Polyfibrospongia) echina* (2). It is of interest that the sponge previously known as *Spongia fenestra* or *Aplysina aurea* was reclassified as *Smenospongia aurea* (Hyatt) by Wiedenmayer (3).

Our research group studied the phospholipid fatty acid composition of the sponges *Verongula gigantea* and *Aplysina (= Verongia) archeri* and concluded that the similarity in their phospholipid fatty acid composition could be used as a chemotaxonomic tool (4). The key similarities in the phospholipids were the presence of predominantly saturated fatty acids, of considerable quantities of 3,7,11,15-tetramethylhexadecanoic (phytanic) acid and of the very long-chain 5,9,23-tricontatrienoic acid, and of considerable amounts of 2-hydroxy fatty acids. The sterol composition of these sponges was also similar, in particular because both sponges contained aplysterol, verongulasterol, and 25-dehydroaplysterol (5,6). However, the question still remains whether the Caribbean sponge *S. aurea* contains a lipid composition similar to other *Aplysina* sponges.

Literature published in this area prompted us to further study other sponges from the genus *Aplysina*, in particular *Aplysina lacunosa* and *Aplysina fistularis*, in search of similar lipid metabolites which could serve as a sound chemotaxonomic base. We also studied the lipids of the sponge *Smenospongia aurea* in order to compare these with the lipids of sponges of the family Aplysinidae. It was likely that *S. aurea* would contain α-hydroxy fatty acids. We also felt that this type of study could also lead to a better understanding of the metabolism of α-hydroxy fatty acids in sponges in the future.

EXPERIMENTAL PROCEDURES

Aplysina lacunosa was collected on May 25, 1989, at a depth of 24 m. *Aplysina fistularis* and *Smenospongia aurea* were collected March 22, 1991, at 12 m near the shelf edge of La Parguera, Puerto Rico. The sponges were placed in dry ice for transportation to the laboratory. They were freeze-dried on a Labconco Freeze Dryer 4.5 (Model 77510; Kansas City, MO). For extraction, approximately 180 g of dry sponge was carefully cleaned of all nonsponge debris and cut into small pieces. Extraction with 600 mL of chloroform/methanol (1:1, vol/vol) yielded total lipids. The neutral lipids, glycolipids and phospholipids (1.5 g) were separated by column chromatography on silica gel (60–200 mesh) using the procedure of Privett *et al.* (7). The sterols were obtained from the neutral lipids fraction by chromatography on a silica gel column using diethyl ether/hexane (1:1, vol/vol) as solvent. The sterol mixtures were recrystallized two times, the first time in MeOH, the second time in CH_3CN. Final separation was achieved using a Waters Associates high-performance liquid chromatography (HPLC) system (M510 pump; Waters 410 differential refractometer), with an Altex Ultrasphere ODS-2 column (25 cm × 4.6 mm i.d.) and methanol as solvent. The phospholipid classes were separated by preparative thin-layer chromatography (TLC) on silica gel G using chloroform/methanol/water (25:10:1, vol/vol/vol) as solvent. The fatty acid methyl esters from phospholipids were obtained by reaction with methanolic hydrogen chloride (8) followed by purification by column chromatography and elution with hexane/diethyl ether (9:1, vol/vol). The resulting methyl esters were analyzed by gas chromatography/mass spectrometry (GC/MS) using a Hewlett-Packard 5995A GC/MS (Hewlett-Packard, Palo Alto, CA) or a Hewlett-Packard 59970 MS ChemStation equipped with a 20 m × 0.32 mm nonpolar fused silica column (Supelco, Bellefonte, PA) with SPB™-1 as the bonded phase. Hydrogenations were carried out in 10 mL of absolute methanol in the presence of catalytic amounts of platinum oxide (PtO_2). ^1H NMR and ^{31}P NMR spectra were recorded on a General Electric GN-300 (300 MHz) spectrometer in $CDCl_3$. Mass spectral data for the key fatty acids studied are presented below.

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Abbreviations: ECL, equivalent chain length; EIMS, electron impact mass spectrometry; FTIR, Fourier transform infrared spectroscopy; GC/MS, gas chromatography/mass spectrometry; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TLC, thin-layer chromatography.

2-Hydroxy-17-methyloctadecanoic acid methyl ester. MS (70 eV) *m/z* (rel. intensity), 328 (M^+, 3.7), 269 ($M^+ - 59$, 9), 159 (2.3), 151 (2.2), 149 (4), 145 (7), 141 (4), 139 (3), 137 (2.5), 132 (2), 129 (3.5), 127 (12), 125 (8.4), 123 (3.6), 113 (9), 111 (18), 109 (11), 103 (13.5), 99 (5.4), 97 (49), 96 (10), 95 (21), 90 (45), 87 (13), 74 (10), 71 (42), 69 (66), 67 (28), 59 (13), 57 (100), 55 (97).

2-Hydroxy-21-methyldocosanoic acid methyl ester. MS (70 eV) *m/z* (rel. intensity), 384 (M^+, 5.2), 325 ($M^+ - 59$, 5), 207 (2), 185 (2), 145 (5), 137 (2.5), 127 (11), 123 (6), 117 (11.5), 111 (21), 109 (15), 103 (11), 97 (48), 96 (16), 95 (27), 90 (36), 85 (23), 83 (53), 81 (29), 71 (57), 69 (60), 67 (29), 59 (12), 57 (100), 55 (99).

2-Hydroxy-22-methyltricosanoic acid methyl ester. MS (70 eV) *m/z* (rel. intensity), 398 (M^+, 5.8), 339 ($M^+ - 59$, 4.2), 207 (0.7), 170 (0.7), 159 (1.3), 156 (1.2), 151 (0.9), 145 (4.9), 141 (2.5), 137 (2.3), 127 (9.6), 125 (6.8), 117 (2.6), 113 (8.2), 111 (18), 109 (10), 103 (10.6), 99 (6.8), 98 (7), 97 (41), 95 (25), 90 (37), 85 (23), 83 (48.6), 81 (26), 75 (81), 71 (53), 69 (55), 68 (14), 67 (25), 57 (100), 55 (84).

2-Hydroxy-22-methyltetracosanoic acid methyl ester. MS (70 eV) *m/z* (rel. intensity), 412 (M^+, 7.5), 353 ($M^+ - 59$, 3.9), 354 (1.2), 207 (0.36), 169 (0.5), 159 (1.3), 156 (1.0), 145 (4.0), 143 (1.1), 141 (2.6), 137 (2.2), 127 (6.6), 125 (5.9), 123 (4.4), 117 (1.7), 113 (6.8), 111 (14.9), 109 (10), 103 (8.7), 99 (4.5), 97 (31), 95 (20.6), 90 (27.9), 87 (7.0), 85 (18.1), 83 (36.6), 82 (11.6), 81 (20.1), 75 (4.6), 74 (5.5), 71 (33.7), 69 (39.7), 67 (19.9), 57 (100), 55 (60).

2-Hydroxy-24-methylpentacosanoic acid methyl ester. MS (70 eV) *m/z* (rel. intensity), 426 (M^+, 5.8), 367 ($M^+ - 59$, 1.9), 207 (2.3), 159 (2.3), 145 (4.6), 143 (2.8), 141 (2.6), 137 (2.8), 127 (9.6), 125 (7.1), 123 (5), 117 (15), 113 (7.6), 111 (19.9), 109 (11), 103 (8), 99 (5.9), 97 (37), 95 (23.6), 90 (30.8), 87 (11.4), 85 (21.8), 83 (43.4), 82 (19.6), 81 (26.7), 75 (8.6), 74 (14.6), 71 (48.4), 69 (56.7), 67 (27.6), 57 (100), 55 (85).

2-Hydroxy-23-methylpentacosanoic acid methyl ester. MS (70 eV) *m/z* (rel. intensity), 426 (M^+, 5.1), 367 ($M^+ - 59$, 1.6), 159 (1.3), 152 (1.0), 145 (3.1), 141 (2.4), 137 (2.3), 127 (6.5), 125 (5.9), 123 (4.4), 117 (7.9), 113 (6.5), 111 (14.6), 109 (9), 103 (7.4), 97 (31.5), 95 (21.3), 91 (3.3), 90 (28), 87 (5), 85 (19.2), 83 (38.2), 82 (12.3), 81 (20.6), 75 (23), 71 (41), 69 (41), 67 (18), 61 (2.3), 59 (6), 57 (100), 55 (64).

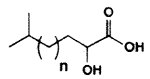
Lithium aluminum hydride reduction. The fatty acid methyl esters (5 mg) were placed in a 50-mL three-necked round-bottomed flask at −78°C in 20 mL of dry THF. Excess $LiAlH_4$ was added and the mixture was left to stir overnight. For the work-up, 10–15 mL of ethyl acetate followed by 10 mL of distilled water were added to destroy excess $LiAlH_4$. The solution was then acidified with H_2SO_4 followed by diethyl ether extraction (2 × 15 mL). The ether extract was dried over anhydrous $MgSO_4$, filtered, and the solvent was evaporated to dryness, affording the corresponding 1,2-diols in almost quantitative yield.

Oxidative cleavage of the 1,2-diols. The 1,2-diols (2–3 mg) were dissolved in 2 mL of *t*-BuOH followed by the addition of 1 mL of a solution of $NaIO_4/KMnO_4$ in distilled water and 1 mL of K_2CO_3 in water. The mixture was stirred vigorously for 1 h, followed by acidification with conc. H_2SO_4. The excess oxidant was destroyed by adding $Na_2S_2O_3$ until the solution remained colorless. The organic extract was taken up with diethyl ether (3 × 4 mL), dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness affording the corresponding fatty acids.

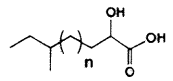
RESULTS

Phospholipid fatty acid compositions of *Smenospongia aurea*, *Aplysina lacunosa* and *Aplysina fistularis* are presented in Table 1. The main phospholipids in the sponges were phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylcholine (PC). PE was the principal phospholipid, while PC was a minor component. ^{31}P nuclear magnetic resonance (NMR) was used to analyze the phospholipid mixture as we did previously (4). The phospholipid fatty acid composition of these sponges represented a series of predominantly saturated fatty acids with considerable amounts of *anteiso* and *iso* acids. Phytanic (3,7,11,15-tetramethylhexadecanoic) acid was present in *ca.* 4% relative abundance in *S. aurea*, 9.4% abundance in *A. lacunosa*, and 9% abundance in *A. fistularis*. The mass spectrum of the phytanic acid methyl ester showed a base peak at *m/z* 101 (100%) and peaks at *m/z* 171 (15%) and *m/z* 241 (2%) indicating branching consistent with its structure (9). A second key fatty acid common to all three sponges was the very long chain 5,9,23-triacontatrienoic acid (30:3). This fatty acid was identified by its retention times in capillary gas chromatography (GC), equivalent chain length (ECL), and by mass spectral comparison with an authentic sample (10).

Each of the three sponges contained α-hydroxy fatty acids (Table 1). *A. lacunosa* contained a series of α-hydroxy acids (10% of the total fatty acid mixture) ranging in length from 15 to 23 carbon atoms, *A. fistularis* contained α-hydroxy acids from 19 to 24 carbon atoms (7% of the total fatty acid mixture), while *S. aurea* showed the most complete series of α-hydroxy acids ranging in length from 17 to 26 carbon atoms (29% of the total fatty acid mixture). These findings substantially extend current knowledge on α-hydroxy acids in sponges (4). In particular, *S. aurea* contained six novel branched α-hydroxy acids which were characterized as 2-hydroxy-17-methyloctadecanoic acid (**1**), 2-hydroxy-21-methyldocosanoic acid (**2**), 2-hydroxy-22-methyltricosanoic acid (**3**), 2-hydroxy-22-methyltetracosanoic acid (**4**), 2-hydroxy-24-methylpentacosanoic acid (**5**), and 2-hydroxy-23-methylpentacosanoic acid (**6**). All contained either *iso* or *anteiso* terminal methyl



(1) n= 13
(2) n= 17
(3) n= 18
(5) n= 20



(4) n = 18
(6) n = 19

branching. Characterization of these acids was by GC retention times, GC/MS, chemical degradation to the known saturated *iso*/*anteiso* saturated compounds, and nuclear magnetic resonance spectroscopy. A representative example is the characterization of 2-hydroxy-21-methyldocosanoic acid methyl ester, which showed a very characteristic MS spectrum with a molecular ion at *m/z* 384 (5%, $C_{24}H_{48}O_3$). The identification of an $M^+ - COOCH_3$ fragmentation ion at *m/z* 325 (5%) strongly

TABLE 1

The Phospholipid Fatty Acids and Aldehydes from the Sponges

	Abundance (%)		
	S. aurea	*A. lacunosa*	*A. fistularis*
Fatty acids			
Methyltridecanoic (14:0)	0.1	—	—
Tetradecanoic (14:0)	0.6	0.9	1.0
Methyltetradecanoic (15:0)[a]	8.0	5.1	14.2
Pentadecanoic (15:0)	1.0	1.5	1.0
Methylpentadecanoic (16:0)[a]	3.0	1.8	11.2
9-Hexadecenoic (16:1)	1.0	—	—
Hexadecanoic (16:0)	7.1	3.6	18.9
Heptadecenoic (17:1)	1.3	3.6	2.8
Methylhexadecanoic (17:0)[a]	12.0	6.9	8.9
Heptadecanoic (17:0)	0.6	1.5	0.8
Octadecenoic (18:1)	1.0	—	—
Methylheptadecanoic (18:0)[a]	0.4	—	—
Octadecanoic (18:0)	4.6	—	3.3
Methyloctadecanoic (19:0)[a]	9.2	—	—
Nonadecanoic (19:0)	0.5	7.0	5.7
3,7,11,15-Tetramethylhexadecanoic (20:0)	4.0	9.4	9.1
Eicosatetraenoic (20:4)	—	10.0	—
Eicosanoic (20:0)	2.4	2.7	1.3
Heneicosanoic (21:0)	0.5	0.9	—
Methyleicosanoic (21:0)[a]	0.2	—	—
Docosanoic (22:0)	1.7	1.5	—
Methyldocosanoic (23:0)[a]	0.8	—	—
Tricosanoic (23:0)	0.5	—	—
Methyltricosanoic (24:0)[a]	1.6	—	—
Tetracosanoic (24:0)	0.5	0.6	—
Methyltetracosanoic (25:0)[a]	3.7	—	—
Pentacosanoic (25:0)	1.1	—	—
5,9-Hexacosadienoic (26:2)	1.0	—	—
5,9,23-Tricontatrienoic (30:3)	1.6	8.2	9.4
α-Hydroxy acids			
2-Hydroxypentadecanoic (15h:0)	—	0.6	—
2-Hydroxy-14-methylpentadecanoic (*i*-16h:0)	—	0.3	—
2-Hydroxyhexadecanoic (16:0)	—	0.6	—
2-Hydroxy-15-methylhexadecanoic (*i*-17h:0)	0.1	0.3	—
2-Hydroxyheptadecanoic (17h:0)	0.3	0.6	—
2-Hydroxy-16-methylheptadecanoic (*i*-18:0)	1.0	—	—
2-Hydroxyoctadecanoic (18h:0)	1.4	1.2	—
2-Hydroxy-17-methyloctadecanoic (*i*-19h:0)[b]	0.4	—	—
2-Hydroxynonadecanoic (19h:0)	1.4	0.6	0.7
2-Hydroxyeicosanoic (20h:0)	5.5	—	2.6
2-Hydroxyheneicosanoic (21h:0)	1.3	1.5	0.9
2-Hydroxydocosanoic (22h:0)	2.1	1.8	1.1
2-Hydroxy-21-methyldocosanoic (*i*-23h:0)[b]	0.3	—	—
2-Hydroxytricosanoic (23h:0)	0.8	2.7	—
2-Hydroxy-22-methyltricosanoic (*i*-24h:0)[b]	1.7	—	—
2-Hydroxytetracosanoic (24h:0)	5.9	—	2.4
2-Hydroxy-23-methyltetracosanoic (*i*-25h:0)	2.1	—	—
2-Hydroxy-22-methyltetracosanoic (*ai*-25h:0)[b]	1.5	—	—
2-Hydroxypentacosanoic (25h:0)	1.6	—	—
2-Hydroxy-24-methylpentacosanoic (*i*-26h:0)[b]	0.3	—	—
2-Hydroxy-23-methylpentacosanoic (*ai*-26h:0)[b]	0.5	—	—
2-Hydroxyhexacosanoic (26h:0)	0.6	—	—
Aldehydes[c]			
Undecanal (11:0)	—	1.2	—
Tridecanal (13:0)	—	0.6	—
Pentadecanal (15:0)	—	0.9	—
Heptadecanal (17:0)	—	1.2	—
Octadecanal (18:0)	0.3	11.0	1.4
Nonadecanal (19:0)	—	1.8	—
Tetracosanal (24:0)	0.5	—	—
Pentacosanal (25:0)	—	2.1	—
Heptacosanal (27:0)	—	5.7	—

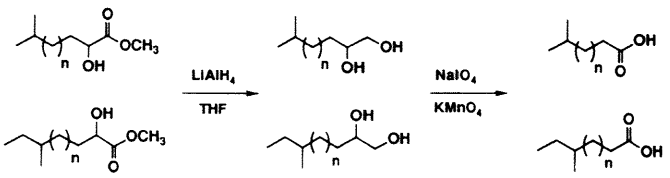
[a] Normally these were mixtures of *iso* and *anteiso* acids.

[b] First reported in a marine organism.

[c] Derived from alk-1-enylacyl phospholipids.

suggested α-substitution. Lack of significant peaks at *m/z* 74 and *m/z* 104, but the presence of fragmentation ions at *m/z* 90 (36%) and *m/z* 103 (11%) derived by McLafferty rearrangement, indicated the presence of the α-hydroxy structure. This was further confirmed by the long retention time in capillary GC with an ECL value of 23.88 found. The Fourier transform infrared (FTIR) spectrum of the mixture displayed a band between 3100 and 3600 cm^{-1}, supporting the presence of a hydroxy group. ^1H NMR of the 2-hydroxy fatty acid methyl esters showed the signal of the methoxy group of the ester at 3.78 ppm, while the signal of the methoxy group of a normal fatty acid methyl ester appears at 3.66 ppm. This downfield shift is consistent with α-hydroxy substitution. Moreover, the signal of the hydrogen at position 2 in the 2-hydroxy fatty acid methyl esters was observed between 4.1 and 4.2 ppm as expected for α-hydroxy fatty acid methyl esters. The identification of the entire series of long-chain α-hydroxy esters from *S. aurea* was possible based on a plot of retention time *vs.* number of carbon atoms. Three different families of α-hydroxy fatty acid methyl esters could thus be discerned.

The methyl branching in the novel α-hydroxy fatty acids was unequivocally determined by chemical degradation to the corresponding known *iso* and *anteiso* non-hydroxylated saturated fatty acids. The sequence used is shown in Scheme 1. The process resulted in a chain shortening



SCHEME 1

of the α-hydroxy esters by one carbon atom, while the non-hydroxylated saturated esters were unaffected. Hydrogenation of the mixture with PtO_2 in MeOH followed by $LiAlH_4$ reduction afforded the corresponding saturated 1,2-diols which were then cleaved and oxidized with $NaIO_4/KMnO_4$ to afford the corresponding nonhydroxylated fatty acid with one carbon less. These acids were then esterified with HCl/MeOH in order to obtain the corresponding fatty acid methyl esters. In all cases, the fatty acid methyl esters thus obtained corresponded to the saturated *iso* or *anteiso* methyl esters with one carbon less as shown by their ECL values as well as by GC coinjection with authentic standards. For example, the 2-hydroxy-22- and 23-methyltetracosanoic acid methyl esters (*ai*-25h:0 and *i*-25h:0) were transformed into the corresponding 23- and 24-methyltricosanoic acid methyl esters (*ai*-24:0 and *i*-24:0) by the sequence of reactions outlined above. The former methyl ester showed an ECL value of 23.63 and the latter an ECL value of 23.72. Similar results were obtained for the 2-hydroxy-23- and 24-methylpentacosanoic acid methyl esters (*ai*-26h:0 and *i*-26h:0) which were also transformed into the corresponding 22- and 23-methyltetracosanoic acid methyl esters (*ai*-25:0 and *i*-25:0) with ECL values of 24.75 and 24.63, respectively. By means of a series of recrystallizations from CH_3CN, we were able to isolate the 2-hydroxytetracosanoic acid methyl ester from *A. fistularis*, thus permitting the measurement of the optical rotation in methanol which was found to be a small positive number ($[\alpha]_D = +4.0°$). Therefore, α-hydroxy fatty acids from sponges seem to belong to the D-series.

A series of saturated aldehydes was also characterized which arose from 1-*O*-alk-1-enyl-2-acyl glycerophospholipids, commonly known as plasmalogens (11). While *S. aurea* produced only two aldehydes, namely octadecanal (18:0) and tetracosanal (24:0), *A. lacunosa* produced a more complete series of aldehydes ranging in length from 11 to 27 carbon atoms and accounting for 25% of the total long-chain components of the phospholipids of this sponge. Heptacosanal (27:0) is the longest aldehyde yet derived from sponge plasmalogens (11). The aldehydes were characterized as their dimethyl acetals. Dimethyl acetals normally do not afford a molecular ion in electron impact mass spectrometry (EIMS), but an ion equivalent to $[M - 31]^+$ (loss of a methoxy group) permits determination of the molecular weight. As an example, heptacosanal dimethyl acetal displayed a $[M - 31]^+$ peak at *m/z* 409 (0.5% abundance) and a base peak at *m/z* 75 ($C_3H_7O_2^+$).

The sterols from the three sponges were also isolated, using reverse-phase high-performance liquid chromatography (HPLC) for the final separation. The particular sterols were characterized by 300 MHz ^1H NMR, MS, and by comparison with authentic samples. The *Aplysina* sponges contained, as their major sterol 24*S*,26-dimethyl-25*S*-cholest-5-en-3β-ol (12). From *A. fistularis*, the following sterols were isolated and characterized: cholesterol (12% of the total sterol mixture), 24-methylcholesta-5,22-dien-3β-ol (5%), 24-methylcholest-5-en-3β-ol (5%), 24-ethylcholesta-5,22-dien-3β-ol (3%), 24-ethylcholest-5-en-3β-ol (29%), (24*R*,25*S*)-24,25-dimethylcholest-5-en-3β-ol (39%), and (24*R*)-24,26,27-trimethylcholesta-5,25-dien-3β-ol (6%). Similar results were obtained for *A. lacunosa* where 24*S*,26-dimethyl-25*S*-cholest-5-en-3β-ol (aplysterol) accounted for 39% of the total sterol mixture. On the other hand, in *S. aurea* we could detect neither aplysterol nor verongulasterol. The sterol mixture of *S. aurea* consisted of cholesterol (61% of the total sterol mixture), 24-methylcholesta-5,22-dien-3β-ol (10%), 24-methyl-cholest-5-en-3β-ol (5%), 24-ethylcholesta-5,22-dien-3β-ol (2%), and 24-ethylcholest-5-en-3β-ol (22%).

DISCUSSION

We observed that sponge phospholipid can contain α-hydroxy fatty acids ranging in length from 15 to 26 carbons. We also showed that sponges, such as *S. aurea*, are able to synthesize unusual very long chain branched α-hydroxy fatty acids and introduce them into their phospholipids. A series of *iso* and *anteiso* α-hydroxy fatty acids from 14 to 17 carbon atoms in length were previously identified in the phosphatidylethanolamines from *Nocardia leishmanii* (13); the 2-position of PE was exclusively substituted by branched α-hydroxy acids, while the 1-position contained chiefly *iso*-C_{16}, *iso*-C_{17}, and *anteiso*-C_{17} acids. A similar pattern was earlier reported for the lipids of *Streptomyces sioyaensis*, where 2-hydroxy-13-methyltetradecanoic acid was the main α-hydroxy acid, which was also found in the β-position in PE (14). A more

recent report showed that 2-hydroxy fatty acids, such as 2-hydroxy-15-methylhexadecanoic acid, are also found in PE from myxobacteria. Essentially two types of PE were encountered: one contained the nonhydroxy fatty acids, while the other contained 50% hydroxy fatty acids next to nonhydroxy fatty acids (15). *S. aurea* showed a similar picture except that *S. aurea* can synthesize longer chain fatty acids.

A second conclusion from our work relates to the chemotaxonomy of the sponges studied here. The phospholipid fatty acid compositions of *A. lacunosa* and *A. fistularis* were found to be quite similar to the ones reported for other sponges belonging to the family of the Aplysinidae, *i.e., Aplysina archeri* and *Verongula gigantea* (4). *S. aurea* also possesses a very similar fatty acid composition, except that more saturated *iso* and *anteiso* fatty acids were found in this sponge. The most interesting finding, however, was the difference in sterol composition between the *Smenospongia* and *Aplysina*. In both *A. lacunosa* and *A. fistularis* we found aplysterol to be the main sterol of both sponges (39% of the total fatty acid composition) and verongulasterol to be a minor constituent. We previously had obtained similar results for *A. archeri* and *V. gigantea* (5,6). *S. aurea*, on the other hand, showed a common sponge sterol composition, and no traces of aplysterol and/or verongulasterol were detected. Therefore on the basis of their sterol composition, the *Smenospongia* and *Aplysina* represent a different genus.

ACKNOWLEDGMENTS

Dr. Vance Vicente from the United States Department of the Interior Fish and Wildlife Service, classified the sponges. This work was supported by the National Science Foundation under Grant No. CHE-8715649 and the National Institutes of Health (NIH-MBRS Program) under Grant No. S06 RR08102-17. N. Carballeira thanks NSF for a two year creativity extension award.

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[Received March 25, 1992, and in revised form June 15, 1992; Revision accepted July 24, 1992]